

A genetic analysis of the determination of cuticular polarity during development in *Drosophila melanogaster*

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SUMMARY

The polarity mutants *pk*, *sple*, *mwh*, *fz* and *in* alter the orientations of cuticular processes in several regions of the body. The mutant polarity patterns are constant and do not result from alterations in cell lineage. Polarity patterns are locus specific rather than allele specific (new alleles express the same polarity patterns as the original alleles). In the wing, polarity formation is largely cell autonomous and is independent of the anteroposterior compartment boundary. By genetic and physiological manipulation it is shown that the mutant polarity patterns are unaffected by the size of the wing blade or the number of cells that form it. Mutants which remove parts of the wing margin or alter the distribution pattern of wing veins do not alter the mutant polarity patterns. Thus, neither the wing margins nor the pattern of vein tissue act as spatial references for polarity formation. The determination of mutant polarity patterns is not dependent on the overall topology of the wing blade but is region-specific. The mutants affect several independent functions. The possible wild-type function of the loci in polarity formation is discussed.

INTRODUCTION

The cuticle of *Drosophila melanogaster* is secreted by an epithelium of one cell layer. The body surface of the adult fly can be considered to be patterned following three distinct criteria:

- (1) Parts of the surface have specific topological shapes. For example, the outline of the notum is different from that of the wing.
- (2) Differentiated elements are arranged in a specific spatial pattern. For example, the chaetae occupy characteristic positions within the notum.
- (3) Most regions of the body surface bear cuticular processes such as trichomes and chaetae. The orientation of these elements is not random but polarized: they therefore define a set of vectors running over the body surface (Piepho, 1955).

One approach to the understanding of morphogenetic processes is to study mutants that alter them. This has been done for mutants affecting the first

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criterion of patterning (for example, Waddington, 1972) and the second criterion of patterning (reviewed by García-Bellido & Ripoll, 1978). Tokunaga & Stern (1969) have studied a mutant that changes both criterion 1 and criterion 3 patterning. In this paper, a series of mutants that affect only the third criterion of patterning independently of the first and second criteria are studied. These mutants are referred to as 'polarity' mutants and the characteristic pattern produced by each as its 'mutant polarity pattern'. In *Drosophila melanogaster*, four mutants known to affect cuticle polarity are: *prickle* (*pk*), *spiny legs* (*sple*), *frizzled* (*fz*) and *inturned* (*in*). These mutants do not cause the wild-type polarity pattern to become variable, but cause specific alternative polarity patterns to be expressed.

The aims of this work are to investigate the mechanisms controlling the formation of wild-type polarity and to try to infer the function of the loci affecting polarity from the behaviour of mutant alleles.

MATERIALS AND METHODS

The mutants used are listed in Lindsley & Grell (1968) except for the cell markers *pawn* (García-Bellido & Dapena, 1974) and *tricornered* (*trc*) (Ferrús, 1976). The cell size mutant *l(3)Me109* is described in Ferrús & García-Bellido (1976). The meiotic map positions of the polarity mutants are *pk* (2-55.3), *fz* (3-41.7) and *in* (3-47), *sple* was re-mapped to 2-56 based on its presence in 2 out of 4 recombinants in the interval between *pk* (2-55.3) and *cn* (2-57.5). The cell marker *pawn* (*pwn*) maps at (2-55.4) between *pk* and *cn*. Thus, the sequence of these loci is either *pk pwn sple* or *pk sple pwn*. They are all contained within Df(2R) 42E3-43C3.

Mitotic recombination was induced using a Philips Be source working at 100 kV, 15 mA with a 2 mm Al filter. Larvae were irradiated with 1000 R. In some experiments, the *Minute*⁺ technique (Morata & Ripoll, 1975) was used to increase the size of clones. Larvae were irradiated at 48-72, 72-96 or later than 96 h after egg laying (h AEL). Larvae irradiated after 96 h AEL were collected as they pupariated and their age at irradiation calculated in hours before puparium formation (h BPF).

To study cell lineage, marked clones were induced in flies homozygous for one of the polarity mutants. The mutants *pwn*, *multiple wing hairs* (*mwh*) and *javelin* (*ju*) were used as cell markers. The genotypes were *pk; mwh/M(3)i⁵⁵*, *sple; mwh ju/+*, *pwn/+*; *fz* and *pwn/+*; *in*.

To study the interaction of mutant and wild-type tissue, 'morphogenetic mosaics' of clones expressing a polarity mutant phenotype were induced in a phenotypically wild-type background. Larvae were heterozygous for a polarity mutant and an associated, recessive, cuticular marker mutant. Thus, induced mitotic recombination gave marked homozygous polarity mutant clones. The exact genotypes are given under *Results*.

We attempted to produce changes in polarity patterns by physiological and genetic manipulations. The physiological parameters altered were temperature and nutrition. For the nutritional experiments, larvae were washed from standard agar medium at 90 h AEL. They were subsequently maintained on 2% sucrose solution (Beadle, Tatum & Clancy, 1938). A small proportion of such starved larvae (< 10%) completed metamorphosis to give miniature adults.

Deficiencies and point mutations were induced using X-rays and ethyl methane sulphonate (EMS). Males were irradiated with 3000 R or treated with EMS under the conditions described by Lewis & Bacher (1968). Males were mated for two days after treatment, to virgin females carrying *pk*, *fz* and *in*, or *sple*, on marked chromosomes (*pr pk cn sp*; *fz in ri p^P/TM3* or, *b pr sple c px sp*).

RESULTS

(I) Description of the polarity mutant phenotypes

The mutant alleles studied alter the polarity patterns in specific regions of the adult cuticle but leave the remainder of the body surface apparently unaffected. They are all recessive. *pk* changes the polarity pattern of wing, notum and haltere. *sple* affects legs, abdominal tergites and abdominal sternites. *in* affects wing, notum, haltere, abdominal tergites and abdominal sternites. *fz* affects wing, notum, haltere, legs, abdominal tergites and abdominal sternites. During the course of this study it became clear that *mwh* also is a polarity mutant in that it produces a mutant polarity pattern in the wing and haltere. In regions of the body affected by the mutants, trichomes and chaetae point along curved vectors which are different from those of the wild-type pattern (see Figs. 1–4). The mutant polarity patterns are highly constant from fly to fly, the only variability that could be detected was within a small area of region D in *fz* and *in* wings (Fig. 1). The ‘strength’ of the polarity phenotype in a given region of the body surface does not correlate with the number of regions affected by a given mutant (Table 1).

In addition to altering cellular polarity, three of the five mutants cause an increase in the number of processes expressed by trichome-bearing cells. In *mwh* flies from three to six trichomes are formed. Unlike the changes in polarity pattern, this occurs over the whole body surface and is known to be autonomous in clones. *in* causes duplication, and occasional triplication, of trichomes. This phenotype has an incomplete cellular penetrance; in the wing, 50–60% of trichome-bearing cells express duplicated trichomes. *pk* causes occasional duplication of trichomes. The effect is restricted to the proximal area of region A of the wing and has a cellular penetrance of 2–3%.

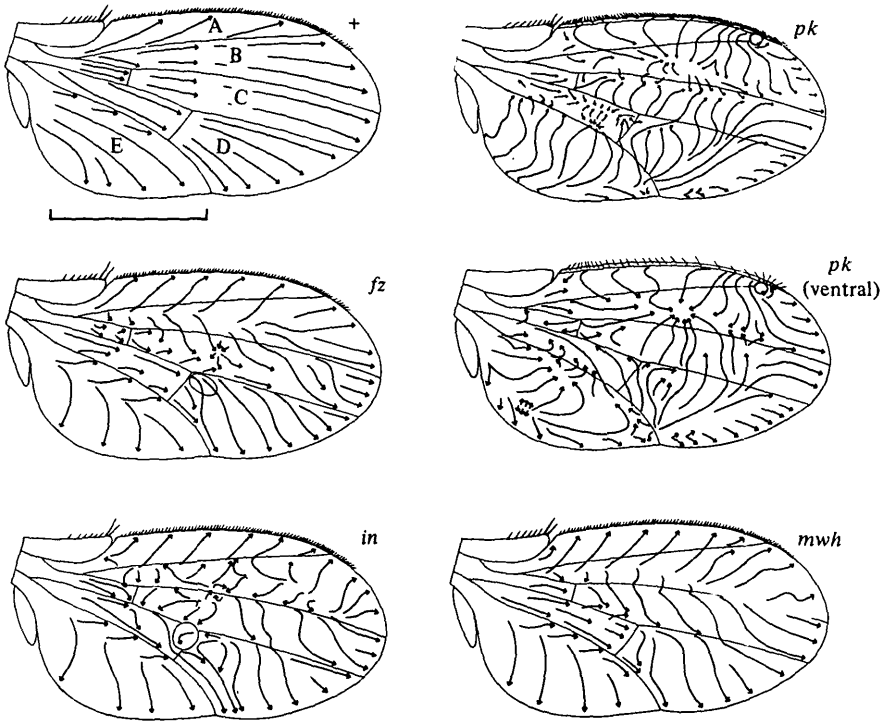


Fig. 1. Polarity patterns on the wing. The patterns on the Dorsal and Ventral surfaces are similar but not completely super-imposable either in wild-type or mutant wings. The Dorsal pattern is drawn except for *pk* where both surfaces are drawn for comparison.

wild-type (+)

The polarity vectors are aligned roughly along the major axis of the wing. Note the nomenclature (A-E) of regions between the wing veins. Bar = 1 mm.

pk

The vectors in marginal areas of the wing are strongly altered. In the anterior wing this alteration reaches the margin and affects the chaetae of the triple row. In the posterior wing the polarity at the margin remains wild type. Near the tip of the 2nd vein there is a whorl which has the same sense of rotation on both surfaces of the wing (anti-clockwise in the right-hand wing and clockwise in the left-hand wing).

mwh in and fz

The vectors are less sharply curved than in *pk* and remain wild type for some distance in from the margin. In *fz* and *in* there is some variability in pattern within the ringed area.

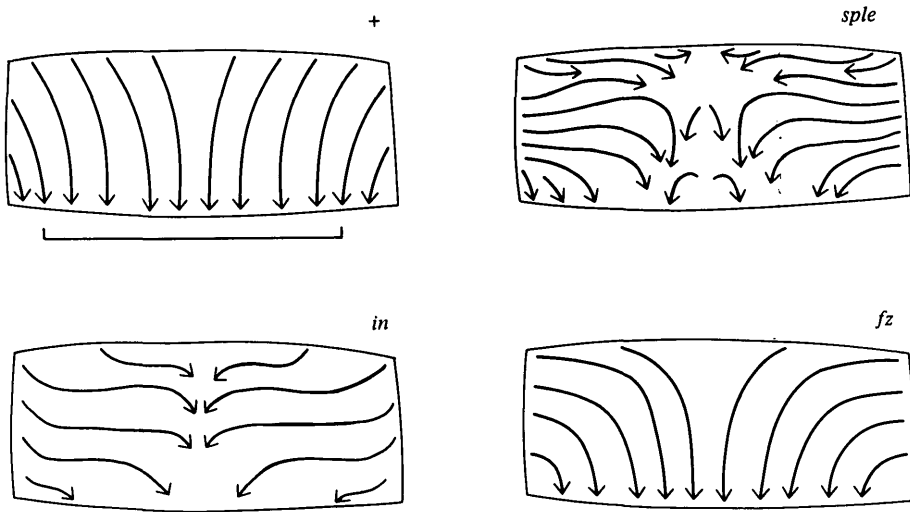


Fig. 2. Polarity patterns in the 3rd abdominal tergites of wild-type (+), *sple*, *in* and *fz*. Bar = 1 mm.

(II) Allelic analysis of the loci

A series of new mutant alleles and genetic deficiencies were isolated in order to define the range of possible phenotypes.

The pk locus

From outcrosses of X-ray mutagenised males to *pk*¹ females, twenty flies expressing *pk*¹ phenotypes were found among 12500 progeny. These showed typical *pk*¹ polarity patterns in wing and notum but wild-type polarity patterns over the remaining body surface. From these flies, thirteen descendent stocks were established.

Five stocks, Df(2R) *pk*^{-78k}, Df(2R) *pk*⁻⁷⁸ⁿ, Df(2R) *pk*^{-78r}, Df(2R) *pk*^{-78s} and Df(2R) *pk*^{-78u}, contain chromosome aberrations that include a visible deficiency in salivary gland squashes. The missing bands that are common to all stocks are 42E3-43C3. When heterozygous over *pk*¹, the deficiency stocks give flies expressing the typical *pk*¹ polarity pattern (see Fig. 5). The deficiency stocks are lethal when homozygous and in heterozygous combinations of one over another. Over a wild-type chromosome Df(2R) *pk*^{-pwn}*sple*⁻ flies express the wild-type polarity pattern. The progeny of outcrosses of these deficiency stocks to *sple* and *pwn* express *sple* or *pwn* phenotypes respectively (*pwn* flies are viable, but sterile, as homozygotes although the original stock contained an associated lethal).

Flies from eight stocks (*pk*^{78a}, *pk*^{78b}, *pk*^{78d}, *pk*^{78f}, *pk*^{78g}, *pk*⁷⁸ⁱ, *pk*^{78m}, and *pk*^{78t}) give wild-type phenotypes when outcrossed to *pwn* or *sple*. Over *pk*¹, or Df(2R) *pk*^{-78k}, these stocks give flies indistinguishable from homozygous *pk*¹

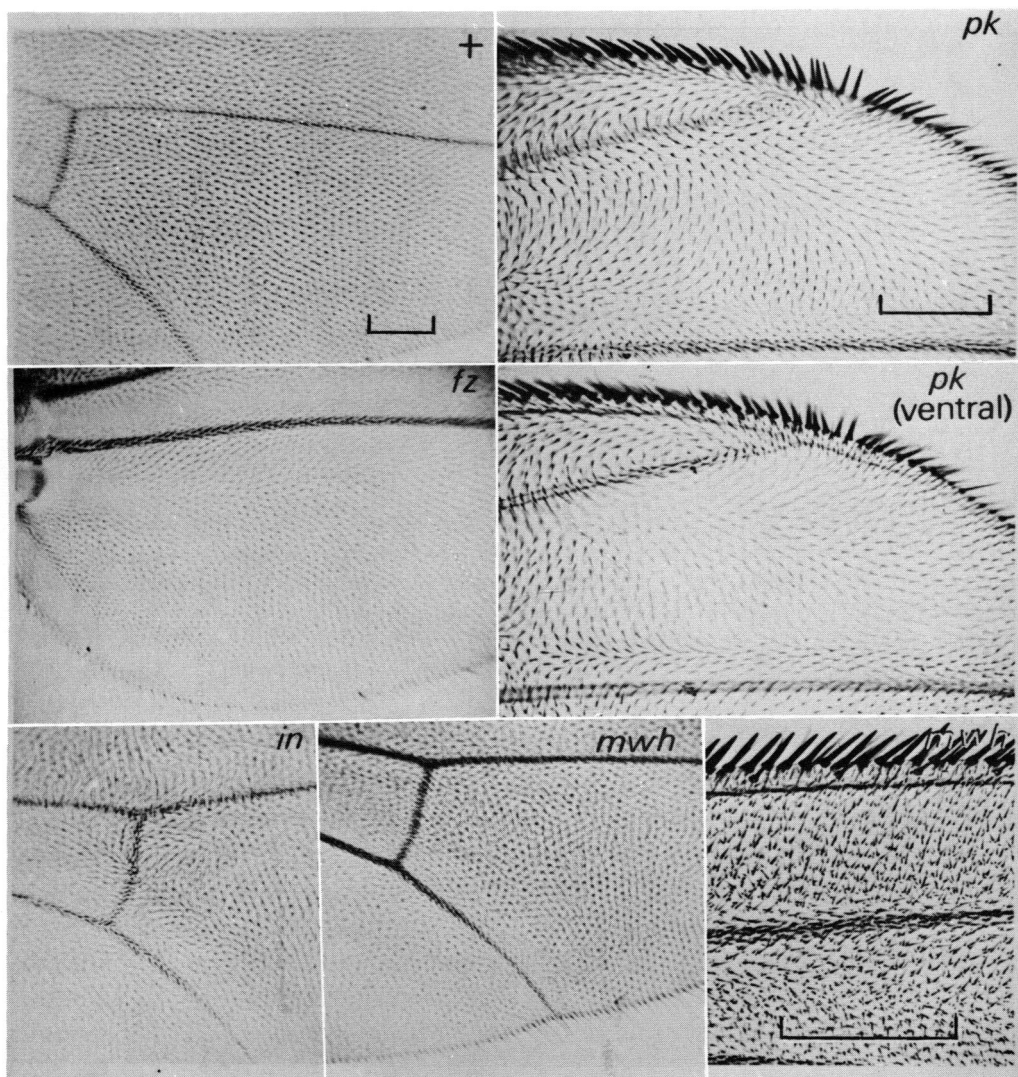
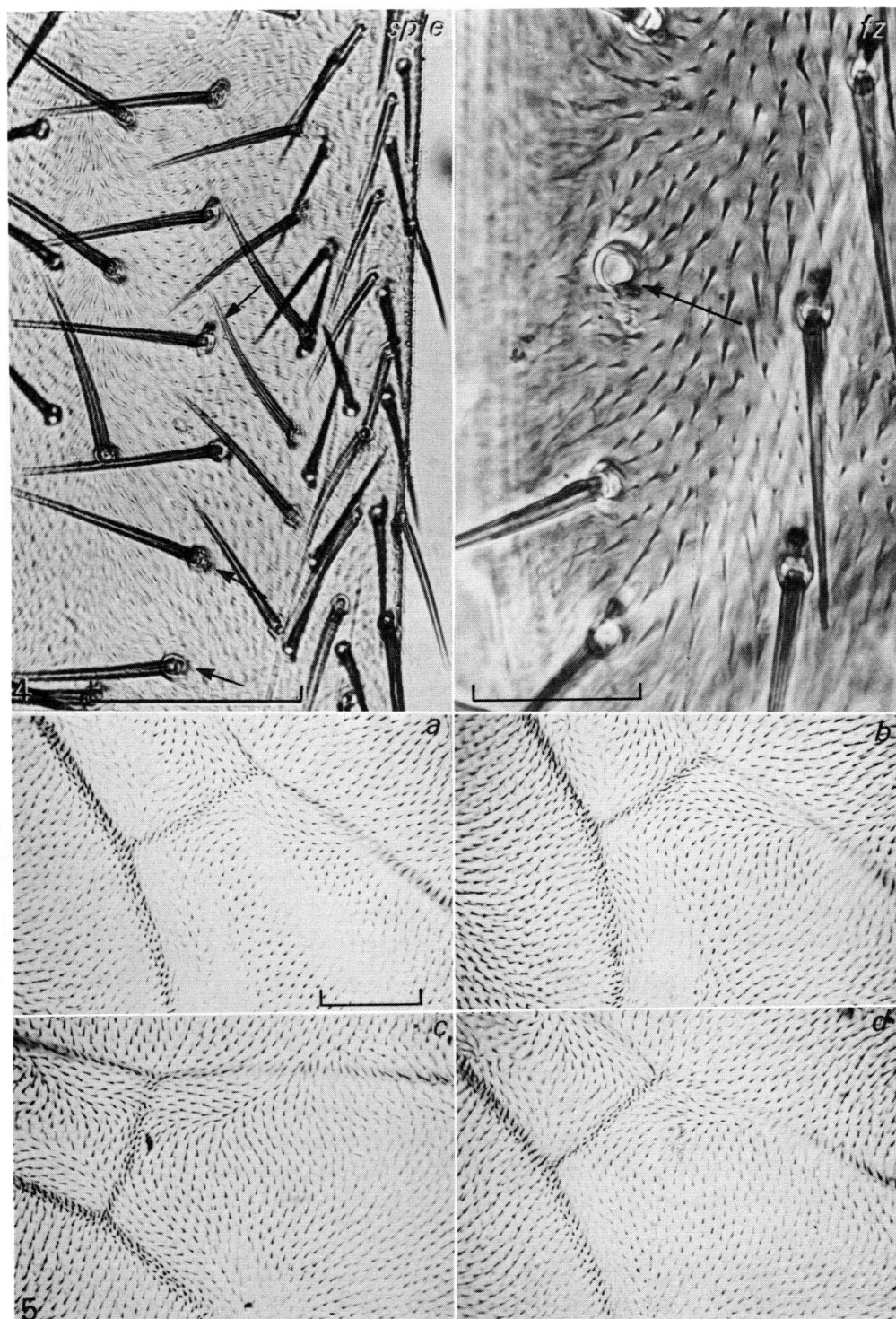


Fig. 3. Polarity patterns in the wing. The directions in which the hairs lie trace out the vectors drawn in Fig. 1.

Fig. 4. Polarity patterns in the legs of *sple* and *fz*. Note chaeta sockets are not radially symmetrical but have a lip, the orientation of which marks the socket polarity. In the wild type many chaeta sockets have associated bracts which point along the axis of the leg; hence expressing the same polarity as the chaeta sockets. In *sple* and *fz* the chaeta sockets and bracts are rotated as a unit (García-Bellido, 1972; Tobler *et al.* 1973). Thus the distribution patterns, as well as the polarity, of bracts is altered. Bar = 100 μ m.

Fig. 5. Polarity patterns of different alleles of *pk*. Note that the patterns are indistinguishable. Bar = 100 μ m.

(a) $pk^1/Df\ 2R\ pk^{-78k}$ (b) pk^{78a}/pk^{78a} (c) $pk^{78a}/Df\ (2R)\ pk^{-78k}$ (d) pk^{78d}/pk^{78d}



flies (see Figs. 1 and 3). That flies of all these stocks are viable when heterozygous with *Df(2R) pk^{-78k}* implies that none of them carry lethal mutations at the *pk* locus. Three stocks contain no lethal mutations on the second chromosome and give homozygous flies. *pk^{78a}/pk^{78a}* and *pk^{78m}/pk^{78m}* flies are indistinguishable from *pk¹/pk¹*. *pk^{78d}/pk^{78d}* flies show a slight alteration to the polarity pattern in region E of the wing, but are otherwise the same as *pk¹/pk¹*.

The sple locus

Two stocks were recovered among 630 progeny after X-ray mutagenesis. The first, *sple^{78a}* gives viable homozygous flies. The second, *Df(2R) sple^{-78b}*, is a deficiency containing the *pk*, *pwn* and *sple* loci. *Df(2R) sple^{-78b}* is lethal when heterozygous with *Df(2R) pk^{-78k}*.

The phenotypes of *sple¹/Df(2R) sple^{-78b}* and *sple¹/Df(2R) pk^{-78k}* are indistinguishable from *sple¹*.

sple^{78a} flies express the *sple¹* polarity pattern in legs and abdomen. Unlike *sple¹*, the polarity patterns in wing and notum are also mutant and the corneal facet packing is disrupted. The phenotype of *sple^{78a}* is indistinguishable from that of the following genotypes: *sple^{78a}/Df(2R) pk^{-78k}*, *sple^{78a}/Df(2R) sple^{-78b}*, *pk¹sple¹/Df(2R) pk^{-78k}*, *pk¹sple¹/Df(2R) sple^{-78b}*, and *pk¹sple¹/pk¹sple¹*. This implies that *sple^{78a}* is a double mutant affecting both loci or possibly a small deficiency containing both loci, although no aberration is visible in salivary glands. That both loci are affected is supported by the phenotype of *sple^{78a}/sple¹* and *pk¹sple¹/+sple¹* being indistinguishable from *sple¹*.

The fz locus

Four stocks were recovered among 5400 progeny after X-ray treatment and four among 3200 after EMS treatment. When heterozygous with *fz¹*, these all show a phenotype indistinguishable from *fz¹*. One mutant stock is viable when homozygous and gives flies indistinguishable from *fz¹*.

The in locus

Three stocks were obtained after X-ray treatment among 5400 progeny. One of these was visibly deficient for salivary gland chromosome bands 76D-77E-F including both the *in* and *ri* loci. All these stocks, in heterozygosis with *in*, give flies indistinguishable from *in*. In addition, one homozygous viable allele was induced with EMS. This gave homozygous flies indistinguishable from *in*.

In summary, the mutant polarity patterns are locus specific. Newly induced alleles express the same polarity patterns as the original alleles. The phenotypes expressed by the different polarity mutant alleles in single dose, i.e. over their respective deficiencies, are the same as the original alleles in homozygosis. This implies that the mutant alleles are amorphs (see Discussion).

In succeeding sections, the original alleles were used as representative of their respective loci.

(III) Clonal analysis

Clonal analysis was used firstly to study the relationship between the mutant phenotypes and clonal growth (cell lineage). Marked clones were induced within a homozygous mutant background. Secondly, the interactions of mutant and wild-type tissue were studied: mutant clones were induced within a phenotypically wild-type background (morphogenetic mosaics).

(1) Cell lineage

There is no indication that clones of cell marker mutants induced within the wings of *pk*, *mwh*, *fz* or *in* (or the abdomen of *sple*) differ in either shape or size from clones expressing the same markers in wild-type wings. For each polarity mutant 25 cell marker mutant clones were examined and drawn. The topology of these clones was compared to that of the same cell marker mutants within a wild-type background. Although it is difficult to be certain that no minor changes occurred, it is clear that the polarity mutants produce no major changes in clonal shape or size. Thus, the mutant polarity patterns do not result from altered clonal growth.

(2) Morphogenetic mosaics

(A) General results

The cells within large homozygous mutant clones express the same polarity as cells in equivalent positions in entirely homozygous wings. The cells within small clones express wild-type polarity. Large clones of *fz* and *pk* generally cause a short range disruption in the polarity of surrounding tissue which may cross the anteroposterior compartment boundary (A/P boundary). However, such clones have no apparent effect on the opposite wing surface. This implies that polarity formation occurs independently in both wing surfaces.

(B) Detailed results

pk. Homozygous clones of *pk pwn* were induced in *pk pwn/Df(2) Mc^{33a}* wings. Forty-three clones containing more than 200 cells (irradiated before 72 h AEL, *M*, Minute rate of development) were examined. These clones express the typical *pk* polarity pattern seen in corresponding regions of homozygous *pk* wings (see Fig. 6) and cause an alteration in the polarity of surrounding tissue. This effect is localized. It does not extend more than five cell diameters from the clone border even with clones of 1000 or 2000 cells (irradiated 24–48 h AEL, *M*, 16 clones containing more than 1000 cells were examined). Fifty-seven clones of 50–100 cells (irradiated 72–96 h AEL, *M*) were examined. These clones show a polarity that is neither *pk* nor wild-type and have no effect

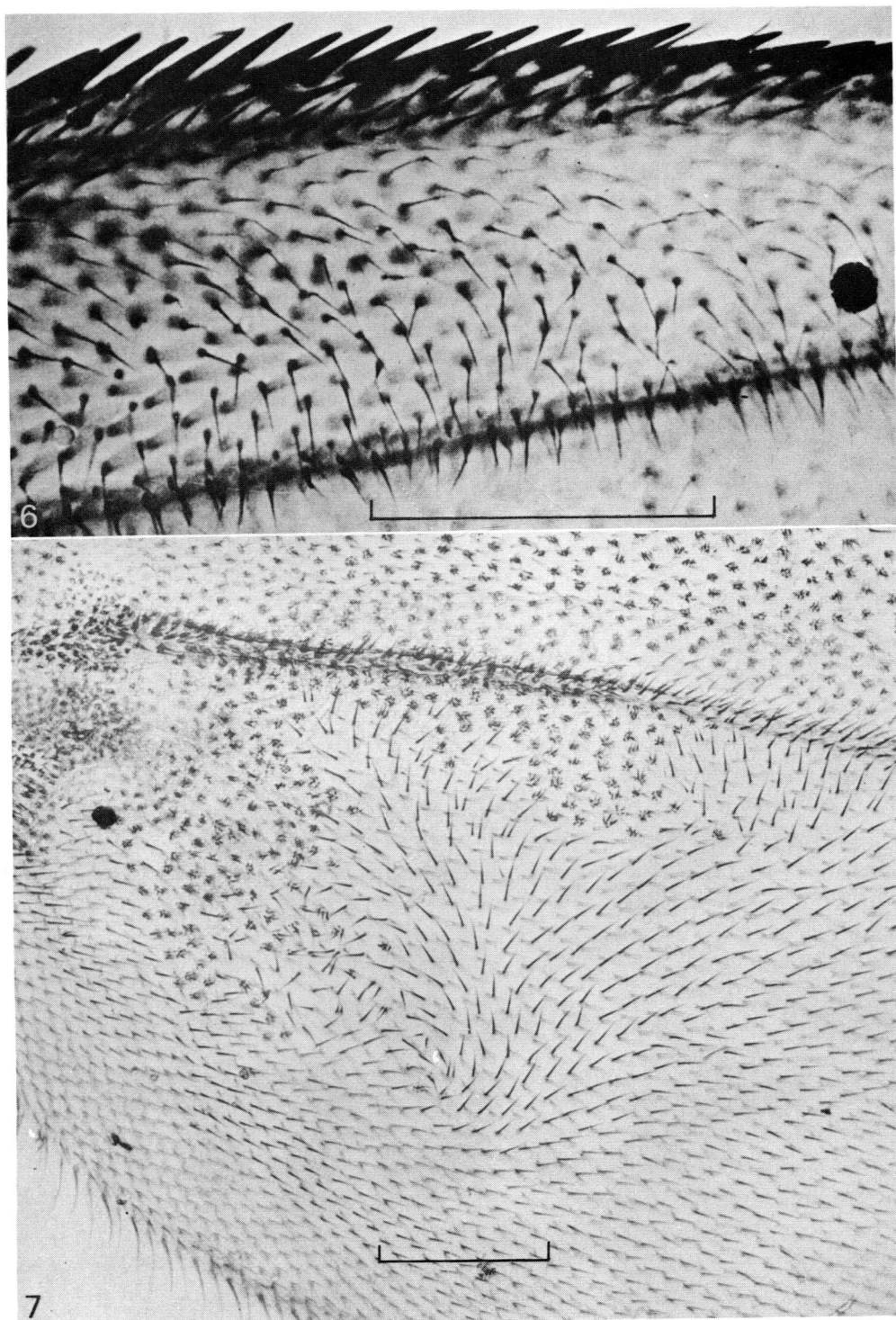


Fig. 6. Large *pk pwn* clone expressing the same polarity as corresponding region of *pk/pk* wing. Bar = 100 μ m.

Fig. 7. Large clone *fz trc* region D and E. $\times 5$. Bar = 100 μ m.

on surrounding tissue. Sixty-one clones smaller than 30 cells (irradiated before 96 h AEL, *M*) were examined. These clones express wild-type polarity.

Fifteen clones larger than 200 cells were found which defined the A/P boundary. In two cases, the disruption in polarity could be seen to cross the boundary. It is difficult to be certain of this point, as the disruption was slight. Clones on one wing surface produce no visible alteration in the polarity pattern on the opposite wing surface. Sixteen clones larger than 200 cells touched the wing margin along the posterior border. However, even in these cases, the disruption of polarity does not cross from one wing surface to another.

mwh. Clones of *mwh* were induced in *mwh/M(3)i⁵⁵* wings. Thirty-seven clones induced by irradiation at 72–96 h AEL, *M* were examined. Clones induced at this stage may fill up to a quarter of a compartment. Such clones express the *mwh* polarity pattern, but cause very little or no effect on surrounding tissue. When present, the disruption extends only 2 or 3 cell diameters into *mwh/M(3)i⁵⁵* tissue.

Fifty clones induced in *mwh/+* wings at 25 h BPF were examined. Clones induced at this stage range from about 10 to 20 cells in size. Clones of 10 cells expressed a wild-type polarity pattern. Clones of 20 cells expressed the *mwh* polarity pattern.

in. Forty-seven clones within *in/M(3)i⁵⁵* wings, induced by irradiation at 48–72 h AEL, *M*, were examined. These clones express *in* polarity. The presence of doubled trichomes is sufficient to mark clones. However, the incomplete penetrance of the trichome doubling effect makes it impossible to define the border of a clone accurately. The only exception is when a clone runs along either the A/P or D/V (dorsoventral) compartment boundaries. Thirteen clones running along the A/P compartment boundary and 27 clones running along the D/V compartment boundary were found. In all cases the polarity of cells on the other side of the boundary was unaffected.

fz. Clones of *fz trc* were induced in *fz trc/M(3)i⁵⁵* flies. It is not possible to observe the polarity within *fz trc* clones in the wing blade as *trc* obscures trichome polarity. Forty-two clones of about 50 cells (72–96 h AEL, *M*) were examined. These have no effect on the polarity of surrounding tissue. Forty-five clones of more than 100 cells were found. Thirteen of these were confined to region A of the wing and cause no disruption in the polarity of surrounding tissue (the nomenclature of wing regions is shown in Fig. 1). The remaining 32 clones alter the polarity of surrounding tissue. The extent of this effect depends on position. Clones confined to regions close to the wing margin produce an alteration extending only 2 or 3 cell diameters into the surrounding tissue. Clones in regions D and E produce an extensive effect (Fig. 7). Twenty-three clones were found that cause a disruption which crossed the A/P compartment boundary (Fig. 8). Twenty-seven clones were found that touched the wing margin but in no case did a disruption cross the D/V compartment boundary.



Fig. 8. Posterior clone of *fz trc* causing a disruption in polarity that crosses the A/P compartment border (dotted line). Bar = 100 μ m.

Fig. 9. *pk en* wing. The anticlockwise whorl associated with the tip of the second vein is duplicated as a clockwise whorl in the transformed posterior wing. Bar = 500 μ m.

(IV) Experimental manipulation of the mutant polarity patterns

Flies were genetically and physiologically manipulated to investigate the extent to which the mutant polarity patterns might be altered.

(a) *Temperature and starvation*

The influence of different temperatures on the development of polarity patterns was studied by comparing flies grown at 17 °C with flies grown at 29 °C. There were no visible differences in polarity patterns either in whole flies or mounted wings (24 mounted wings of each polarity mutant at each temperature were compared).

The polarity patterns expressed in miniature *pk*, *fz* and *in* wings produced after larval starvation were identical to those found in well-fed controls. In the most extreme cases, the wing surface was reduced in area to 50 % and the number of cells to 80 % of well-fed controls. Thus, cells express the mutant polarity typical of their relative positions within the wing. This they are able to do independently of the size of the wing blade or the number of cells that form it.

(b) *Morphogenetic mutants*

Morphogenetic mutants were used to try to identify which elements of the pattern of the developing wing disc might influence polarity formation. A minimum of 30 wings of each genotype were mounted and examined under the microscope.

Wing size

Large flies were produced to investigate the effect of increasing wing size on the expression of mutant polarity patterns. Large wings were produced using the mutant *giant* (*gt*). From counting trichomes, it is clear that the increase in wing size results from increase in cell size not from increase in cell numbers (unpublished results).

The mutant combinations *gt/gt^{E6};fz* and *gt/gt^{E6};in* give flies with an increase in wing area of 20 %. The combination *gt/Df(1)62g¹⁸*, *gt⁻;pk* gives an increase of 70 %. In flies of these genotypes, the mutant polarity patterns of *pk*, *fz*, and *in* remain invariable. The patterns are enlarged to fit the enlarged surface area of the wing, but are otherwise unchanged.

To investigate the interaction of large and small cells during polarity formation, clones of *pk; mwh jv 1(3)Me109* were induced with *pk;mwh jv 1(3)Me109/M(3)i⁵⁵* larvae. In whole flies, *1(3)Me109* is a recessive lethal. It is, however, homozygous viable in clones and causes a cell autonomous increase in surface area of about 30 % (Ferrús, 1976). Larvae were irradiated at 48–72 or 72–96 h AEL, *M*. Twenty-three flies having *pk;mwh jv 1(3)Me109* clones filling between 20 and 60 % of a hemithorax were found. In all cases typical

pk polarity was expressed by both large and normal cell-sized tissue without any disruption at the clone borders. In the wing, clones do not affect the *pk* polarity of surrounding tissue unless they are associated with alterations in the shape of the wing blade. This only occurs when clones larger than 200 cells touch the wing margin.

Wing shape

The effect of changing the topological shape of the wing was investigated using the mutant *lanceolate*² (*ll*²) which increases the length and decreases the width of the wing. *ll*² wings contain more cells along the proximodistal axis and fewer cells along the anteroposterior axis than wild-type wings (unpublished results based on counting trichomes). The total number of cells remains constant, or at least similar.

In *ll*²*pk*, *ll*²*fz* and *ll*²*in* wings the mutant polarity patterns are uniformly distorted to fit the *lanceolate*-shaped wing, but no other changes in polarity occur.

Wing scalloping

The effect of changing the position of the wing margins was investigated using 'scalloping' mutants. Such mutants remove pieces of tissue from the edges of the wing blade. The mutants *Beadex*^J (*Bx*^J), *cut*⁶(*ct*⁶), *clipped* (*cp*) and *Serrate* (*Ser*) were used. In the mutant combinations (*Bx*^J / + ; *pk*, *ct*⁶ ; *pk*, *pk* ; *Ser*, *pk* ; *cp*, *Bx*^J ; *fz*, *Bx*^J / + ; *fz*, *ct*⁶ ; *fz*, *fz* ; *cp*, *Bx*^J ; *in*, *Bx*^J ; *in* and *cp* ; *in*) the presence of a scalloped border hardly affects the polarity pattern expressed by the remaining tissue. The only observable effect is a slight disruption that extends two or three cell diameters from the scalloped border. This effect is also seen in scalloped wings that are not expressing a polarity mutant phenotype.

In summary the polarity patterns expressed by the polarity mutants are unaffected by removing parts of the wing blade.

Wing chaetae and wing vein tissue

In the wild-type wing, chaetae occur only along the wing margins. The mutants *hairy* (*h*) and *Hairy wing* (*Hw*) cause extra chaetae to differentiate within the wing blade. In *pk* ; *h*, *mwh* *h*, *Hw* ; *fz* and *h in* the polarity of the extra chaetae is the same as that normally expressed only by the trichomes. The extra chaeta sockets express an identical polarity to that of the surrounding trichomes and do not cause even local disruptions. Thus altering the distribution pattern of chaetae within the wing blade does not affect the expression of the polarity mutant phenotypes.

This result also demonstrates that the chaetae of the triple row in *mwh*, *fz* and *in* flies express wild-type polarity not because these mutants do not affect

trichomes but, because they do not alter the wild-type polarity patterns of marginal areas of the wing. Trichomes and chaetae express the same polarity.

Two types of mutants that alter the distribution pattern of differentiated wing tissues were used. Firstly, *cubitus interruptus* (*ci*) and *radius incompletus* (*ri*) which remove specific veins, and second, *plexate* (*px*) which causes disruption of the position of the veins and extra vein tissue (García-Bellido, 1977). Two alleles of *px* were used, *px*¹ and *px*⁷². *px*⁷² is an extreme allele which causes large plexae of extra vein tissue associated with slight distortion of the shape of the wing.

None of these mutants alter the expression of *pk*, *mwh*, *fz* or *in* except for a disruption extending up to ten cell diameters from the truncated tips of the fifth vein in *fz;ci* wings. This effect was absent in *pk;ci* and *in;ci* wings.

Minutes

In addition to showing the rate of development, *Minute* mutants alter the expression of many hypomorphic alleles of other genes. The expression of the polarity mutants is unchanged by *Df(2)Mc*^{33a} or *M(3)i*⁵⁵.

Homeotic mutants

Mutants causing homeotic transformations were used to change the shape of compartments and the distribution pattern of differentiated cell types within them.

The mutant *engrailed* (*en*) converts the posterior compartment of the wing into a structure resembling a mirror image of the anterior compartment. (García-Bellido & Santamaria, 1972; Morata & Lawrence, 1975). The expression of *en* is partial and variable. The transformed posterior compartment has an altered shape. It contains specifically anterior structures such as the triple-row chaetae and, sometimes, a duplicated second vein.

The polarity pattern of the transformed posterior compartment in *pk en*, *en;fz* and *en;in* wings is altered and variable. In the distal region of *pk en* and *en;fz* wings these alterations can extend up to ten cell diameters into the anterior compartment (i.e. to within ten cell diameters of the third vein). This effect was seen in 38 out of 124 *pk en* wings and 14 out of 30 *en; fz* wings. Other than this short-range effect, *en* has no effect on the mutant polarity patterns in anterior wing compartments. Thus, the determination of the anterior mutant polarity patterns is not dependent on the overall topology of the wing but is region-specific.

In 13 out of 124 *pk en* wings the tip of the second vein is clearly duplicated in the transformed posterior compartment. When this occurs the whorl associated with the tip of the second vein is duplicated as a clockwise whorl on both wing surfaces (Fig. 9). Thus the posterior whorl in the polarity pattern is a mirror image of that found in the anterior compartment. The duplicated whorl is always either present or absent on both wing surfaces.

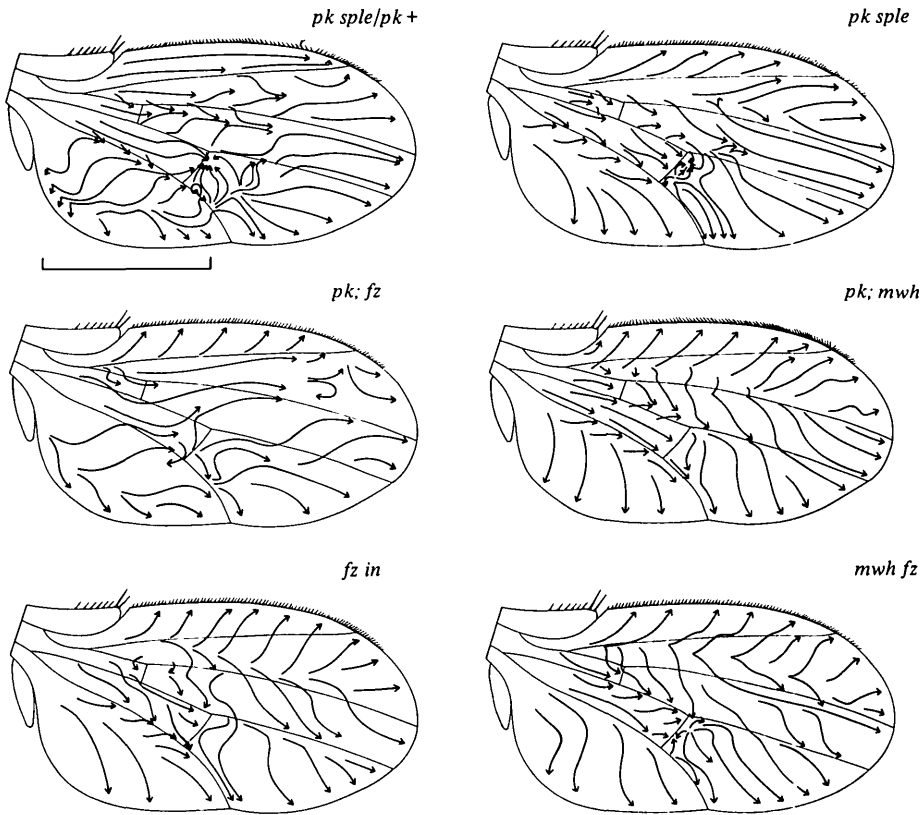


Fig. 10. Wing polarity patterns of double combinations of polarity mutants.
Bar = 1 mm.

The combination of *bithorax*³/*Ultrabithorax*¹³⁰ (*bx*³/*Ubx*¹³⁰) causes transformation of the anterior haltere compartment into anterior wing. In *pk;bx*³/*Ubx*¹³⁰, the homeotic anterior wing compartment expresses a polarity pattern indistinguishable from that of the mesothoracic anterior wing compartment. Thus, it is possible to construct the *pk* wing polarity pattern in an anterior compartment without communication with a normal posterior wing compartment.

(V) Double combinations of polarity mutants

Analysis of combinations of the polarity mutants were undertaken to investigate whether the loci correspond to successive steps in a single pathway or whether independent pathways affecting polarity formation exist. All ten possible pairs of mutants were made. At least 30 wings of each genotype were mounted and examined under the microscope.

Flies carrying a pair of polarity mutants express wild-type polarity when both loci are heterozygous. The expression of one homozygous mutant is

Table 1

Genotype	Wing	Notum	Abdominal tergites	Leg	Corneal facet packing
(a) Different polarity mutants					
<i>pk</i>	m	m	+	+	+
<i>sple</i>	+	+	m	m	+
<i>mwh</i>	m	+	+	+	+
<i>fz</i>	m	m	m	m	m
<i>in</i>	m	m	m	+	+
(b) Pairs of polarity mutants					
<i>pk sple</i>	<i>pk</i> >	<i>pk</i> >	<i>sple</i>	<i>sple</i>	Disrupted
<i>pk;mwh</i>	<i>mwh</i> <	<i>pk</i>	+	+	+
<i>pk;fz</i>	<i>pk</i> > <i>fz</i>	<i>pk</i> > <i>fz</i>	<i>fz</i>	<i>fz</i>	<i>fz</i>
<i>pk;in</i>	<i>in</i>	<i>in</i>	<i>in</i>	+	+
<i>sple;fz</i>	<i>fz</i>	<i>fz</i>	<i>sple</i> > <i>fz</i>	<i>sple</i> > <i>fz</i>	<i>fz</i>
<i>sple;in</i>	<i>in</i>	<i>in</i>	<i>sple</i> > <i>in</i>	<i>sple</i>	+
<i>sple;mwh</i>	<i>mwh</i>	+	<i>sple</i>	<i>sple</i>	+
<i>mwh fz</i>	<i>mwh</i> > <i>fz</i>	<i>fz</i>	<i>fz</i>	<i>fz</i>	<i>fz</i>
<i>mwh in</i>	<i>in</i> > <i>mwh</i>	<i>in</i>	<i>in</i>	+	+
<i>fz in</i>	<i>in</i> > <i>fz</i>	<i>in</i> > <i>fz</i>	<i>in</i> > <i>fz</i>	<i>fz</i>	<i>fz</i>

(a) Regions of the body surface affected by the different polarity mutants. The 'strength' of the mutant phenotype in a given region does not correlate with the number of regions affected; e.g. the polarity vectors in the wings of *fz* are less sharply curved than those of *pk* although *fz* affects more regions of the body than *pk*. Similarly, *sple* affects only abdomen and legs but produces a more sharply curved polarity pattern in these regions than *fz*.

Body regions are marked as having mutant (m) or wild-type (+) polarity.

(b) Regions of the body surface affected by pairs of polarity mutants. Regions are marked as having the pattern expected from the empirical rules given in the text; e.g. *pk* or *pk* > *fz*. Exceptions to the empirical rules are in bold type and mentioned in the text.

unaffected by another heterozygous mutant. There are two exceptions to this; *pk sple/pk* + and *pk/pk;mwh* + which express wing polarity patterns in which the vectors follow slightly different paths to *pk*.

The polarities expressed in double homozygous flies generally obey the following empirical rules:

(1) Regions unaffected by either mutant alone retain wild-type polarity in double mutant flies. (No exceptions).

(2) Regions affected by only one of a pair retain that polarity in double combinations. (1 exception: the thorax and wings of *pk sple* flies express a polarity pattern in which the polarity vectors are less strongly curved than *pk*.)

(3) Regions affected by both of a pair express an intermediate, but invariable, polarity. (2 exceptions: *pk;mwh* wings express *mwh* polarity except at the anterior wing margin where the triple row chaetae express *pk* polarity. Similarly, *pk;in* flies express *in* polarity in both wings and thorax).

These results are summarized in Table 1 and illustrated in Fig. 10.

DISCUSSION

(1) *The determination of polarity*

Cell lineage analysis shows that the parameters of clonal growth in a polarity mutant background are indistinguishable from those in a wild-type background. Thus, the mutant polarity patterns do not result from changes in cell lineage. This implies that the wild-type process of polarity formation is independent of cell lineage.

In morphogenetic mosaics, the cells within large homozygous polarity mutant clones express the polarity typical of equivalent regions in homozygous wings. Thus, the expression of mutant polarity within a large clone surrounded by phenotypically wild-type tissue is cell autonomous. There are two exceptions to this suggesting that the expression of polarity is not completely cell autonomous:

(1) Small clones of any of the polarity mutants express wild-type polarity.

(2) There is a short-range disruption of the wild-type polarity extending into the tissue surrounding large homozygous clones of *pk* or *fz*.

The high degree of cellular autonomy in the expression of polarity implies that polarity formation is region-specific within the wing. If cellular communication is required for the determination of the *mutant* polarity patterns then the interactions of cells are restricted to their close neighbours.

Mutant polarity patterns are not affected in combination with morphogenetic mutants causing scalloping of the wing blade (*Bx*, *ct*, *cp*, *Ser*), alteration of the venation pattern (*ri*, *ci*, *px*, *px⁷²*) or the insertion of extra chaetae within the wing blade (*h*, *Hw*). The stages during which these mutants alter morphogenesis all occur towards the end of the larval development (Santamaria & García-Bellido, 1975; García-Bellido, 1977). Thus, if polarity determination occurs during a final stage in development, i.e. within the puparium, it does not take place with reference to the position of the normal wing margins or the distribution pattern of chaetae or vein tissue. Further, the scalloping mutants do not alter the polarity patterns in the surviving wing tissue implying that no long-range cellular interactions are required late in development.

It is possible that the polarity of cells within the early wing disc might be determined before the stages when the morphogenetic mutants alter morphogenesis, that is, several cell divisions before cuticular differentiation takes place (García-Bellido & Merriam, 1971). In this case, the topology of the early wing disc, or the distribution pattern of some particular cell type within it, might act as a frame of reference for polarity formation. Cells arising in subsequent cell divisions could then take polarities intermediate between those of their close neighbours.

The phenotype of combinations of polarity mutants with homeotic mutants supports the idea of local polarity determination. Within the abnormal composite structures of *en* and *bx³* flies, the mutant polarity patterns of the anterior

compartment are indistinguishable from those expressed in the corresponding regions of normal wings. In the transformed posterior compartment of *pk en* wings, the anti-clockwise whorl associated with the distal tip of the second vein is sometimes duplicated as a clockwise whorl. The duplicated whorl is only expressed when the distal tip of the second vein is also duplicated. This is further evidence that cells autonomously express region-specific polarity patterns. These results again imply that neither the shape nor the distribution of differentiated cell types within the wing blade act as a reference system for polarity formation.

However, the localized disruption of wild-type polarity in tissue surrounding *pk* and *fz* clones implies that the determination of polarity may involve short-range cellular interactions. The determination of polarity is independent of the A/P compartment boundary as the disruption surrounding *fz* clones can cross this boundary.

(2) *The wild-type function of the polarity mutant loci*

The mutant polarity patterns studied are different from each other but invariable within flies of the same genotype suggesting that the wild-type function of the loci might be to control specific aspects of cellular polarity. On this assumption it is interesting to see how different alleles at a given locus might affect the polarity patterns.

At each polarity mutant locus, mutagenesis should be capable of producing a set of recessive alleles in which the wild-type gene product is, partially or completely, inactivated. The possible range is from zero activity to nearly wild-type (Muller, 1933; Falk & Nash, 1974). Flies carrying a given allele over its deficiency will express half the quantity of active gene product as flies homozygous for that allele. The only exceptions would be alleles causing complete lack of active gene products. Flies carrying such alleles would express the same phenotype when homozygous as when heterozygous with their deficiencies. Thus, combinations of a set of alleles with each other and with deficiencies should produce a set of different quantities of gene products with phenotypes ranging from very extreme to nearly wild-type (Muller, 1933).

In the polarity mutant loci studied, however both the original and the newly induced alleles give the same phenotypes when tested over deficiencies or when homozygous. Alleles producing new polarity patterns would have been detected by the screen but were not found. There are two possible explanations. The first is that all the alleles cause complete lack of active gene products. The second is that a threshold exists: flies expressing any below-threshold quantity of active gene product would have the same mutant phenotype. In this case, mutational events leading to alleles with a reduced, but still above-threshold, activity would have been undetected by the screening method. The present data are not sufficient to distinguish between these alternatives.

The lack of correlation between the number of regions affected by a given locus and the degree of curvature of the polarity vectors within the affected regions shows that the loci have some regional specificity. It is not simply that polarity formation is more sensitive to perturbation in some regions than others. That the polarity patterns are locus-specific suggests that they might affect different pathways of polarity formation. The mutant phenotypes could be caused either by a direct or an indirect perturbation of such pathways. If the loci control steps in polarity formation then the mutant phenotypes result from a direct perturbation. In this case the wild-type function of the *pk* locus, for example, is to prevent the wing and thorax expressing *pk* polarity. Lack of *pk*⁺ activity does not prevent polarity formation but, causes the wild-type polarity pattern to be replaced by a more complex mutant polarity pattern. Alternatively, the loci might not control steps in polarity formation but, cause an indirect perturbation to the wild-type pathway leading to polarity formation.

If the polarity mutant loci control sequential steps in a common pathway then flies homozygous for two polarity mutants should express the polarity pattern corresponding to the locus higher in the pathway. This argument makes the assumption that mutant alleles make no, or below-threshold, quantities of active gene product. Alternatively, if the loci do not control steps in a common pathway then new polarity patterns would be expected. These patterns could be: intermediate between those of the two mutants, more extreme than either, less extreme than either, affecting regions of the body unaffected by either mutant alone or, variable or random polarity. On these criteria, the loci do not control steps in a common pathway. The only exceptions are that *mwh* and *in* appear to correspond to higher steps in separate pathways that both lead to *pk*. Thus, although *mwh in* wings express a polarity pattern intermediate between those of *mwh* and *in*, the wings of *pk;in* flies express *in* polarity and those of *pk;mwh* flies express *mwh*, except for the chaetae of the triple row.

Some regions of the body surface are apparently unaffected by any of the known polarity mutants. If the wild-type function of the polarity mutant loci is directly involved in polarity formation, then there may be many similar loci in the genome that have not been detected by mutation. These loci would affect the remaining regions. This would imply that polarity formation is controlled by a complex interaction of pathways. An alternative possibility is that polarity formation is not under the genetic control of a specific developmental pathway. Normal polarity formation could result from the interaction of a set of gene products controlling cellular functions that are only indirectly related to polarity. It could well be a general property of morphogenetic mutants, that their phenotypes result not from specific alterations to a defined pathway but from a constant response of the developing system to a non-specific perturbation. Although a set of loci exists which alter polarity patterns

it does not follow that polarity formation must occur as a discrete developmental process.

This work was supported by a Royal Society European Programme Fellowship and a grant from the British Council. We would like to thank Drs Pedro Ripoll and Gines Morata for many discussions, Dr M. Ashburner and V. Velissariou for analysing the cytology of the deletions. For critical reading of the manuscript: Drs G. Morata, P. Lawrence, J. Karlsson and V. Walker.

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(Received 10 February 1981, revised 9 November 1981)

